<u>Characteristics of Risk of Perinatal Carcinogenesis</u>

<u>by Lucy M. Anderson, Ph.D., D.A.B.T</u>

[The views expressed in this presentation are those of Dr. Anderson and not of the National Cancer Institute]

Thank you. I would like first to congratulate the organizers on this very interesting and informative meeting. Recently there has been considerable discussion about whether certain childhood cancers are increasing in the United States, and whether these are caused by the environment. I have felt the need to step back a bit and ask a more general question: Do we really know that childhood cancers are caused or potentiated by external factors; or, are they just very unfortunate, random genetic accidents and should we be focusing our attention on treatment rather than on risk?

ARE SOME CANCERS IN CHILDREN CAUSED/POTENTIATEDBY EXTERNAL FACTORS?

KNOWN PERINATAL CARCINOGENSIS RISK FACTORS:

1. Transabdominal diagnostic X-rays

2. Diethylstilbestrol

3. Chemo- or radiotherapy in childhood

*MANY OTHER FACTORS STUDIED, INCONSISTENT RESULTS

- No other risk?

- Complex or multiple risks not amenable to epidemiological analysis, because of relatively few case, etc.?

- Epidemiology not asking the right questions?

We know that the human is susceptible to perinatal carcinogenesis because there are three well-established scenarios where this has occurred: Transabdominal x-rays leading to leukemia in children, diethylstilbestrol (DES) leading to adenocarcinoma of the vagina, and secondary cancers after therapeutic treatment with genotoxic agents in childhood. These have been known for decades, and since then there have been many other factors that have been studied, sometimes with positive results and sometimes not, thus, the outcomes have been rather inconsistent.

Does this mean that there are not other risks, or that there are complex and multiple risks that epidemiology is having a hard time picking up because of the low numbers and so forth? Or, is epidemiology not asking the right questions?

The classical way of looking at this type of an issue is to compare incidences of cancers in people of the same race living in very different environments. This is, after all, how we came to know that cancers of the breast, the stomach, the prostate, and the liver, for example, are highly influenced by external factors.

INCIDENCE OF CHILDHOOD CANCER IN
BLACK CHILDREN AFRICA VS U.S.A.

Africa - Algeria
- Mali
- Nigeria
- Uganda
- Zimbabwe

USA - Greater Delaware Valley
- Los Angeles
- New York
- SEER (Connecticut, Atlanta, Detroit, Iowa, New Mexico, Utah, San Francisco, Seattle, Hawaii)

Caveat: In Africa, incaccurate population estimates and possible/probable under-reporting

Parkin et.al., *International Incidence of Childhood Cancer, **Vol. II, 1998***

As a first attempt at this topic, I have used the data from Parkin's new overview on the international incidence of childhood cancers, and simply averaged numbers taken from five widely-scattered countries in Africa for childhood cancer in black children compared with four different datasets for black children in the United States. I did not normalize the data in any way. Furthermore, there are several caveats. In Africa there are probably inaccurate population estimates. Also, there is possible, if not probable, underreporting of cancer cases. This information is suggestive at best, but the outcome is really quite interesting.

Cancer in Black Children
Africa vs U.S.A.
No Differences
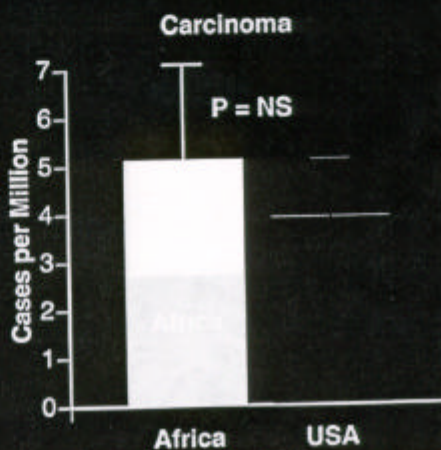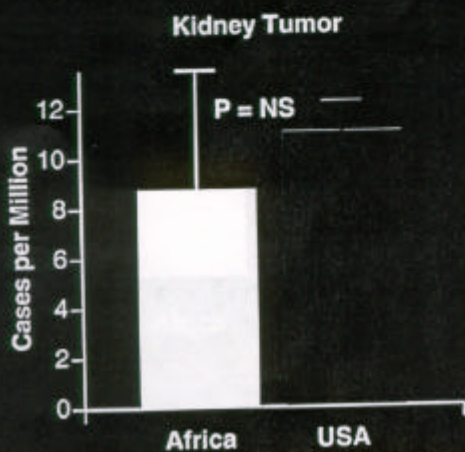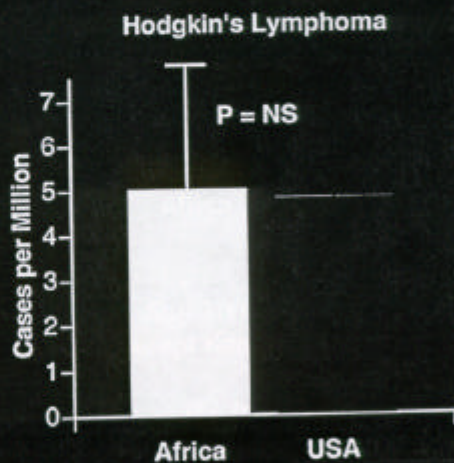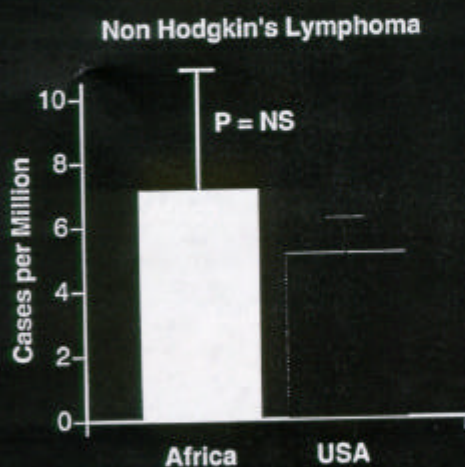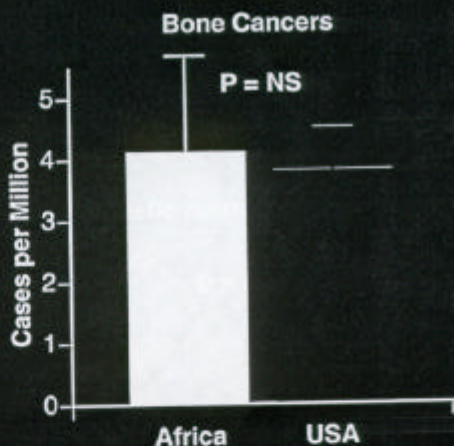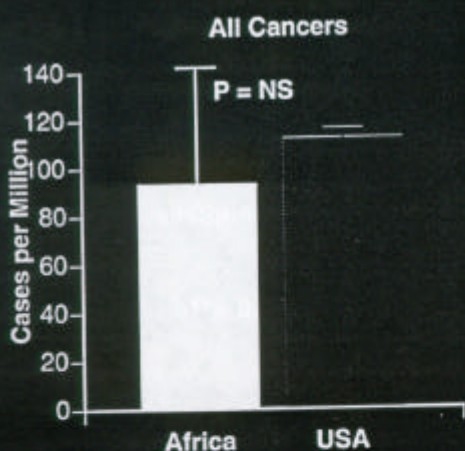
Cancer in Black Children
Africa vs U.S.A.
Those More Common in U.S.A.

Brain/Spinal Tumors

P = 0.0022
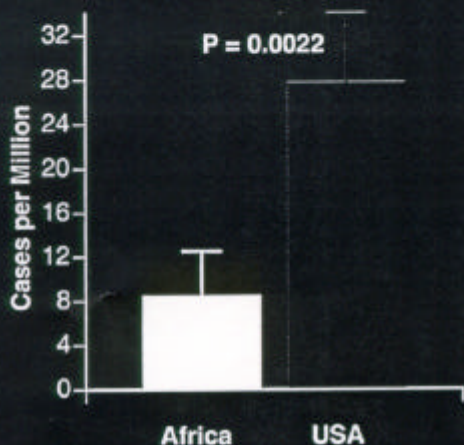
Sympathetic-neuroblastoma Tumors

P = 0.005

Leukemia

P = 0.03
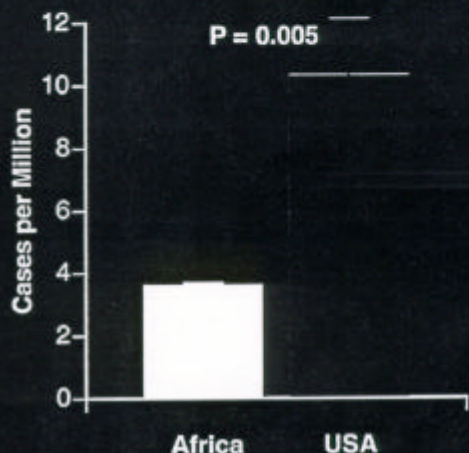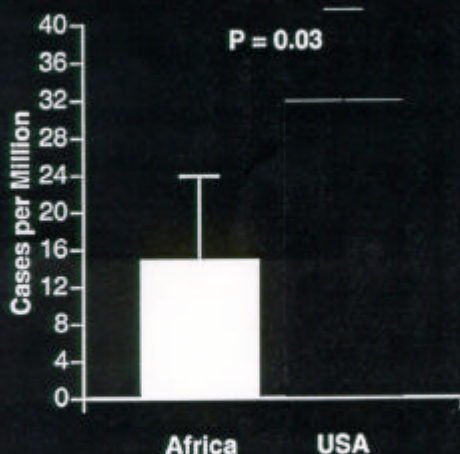
Soft Tissue Cancers
Except Kaposi's Sarcoma

P = 0.0018
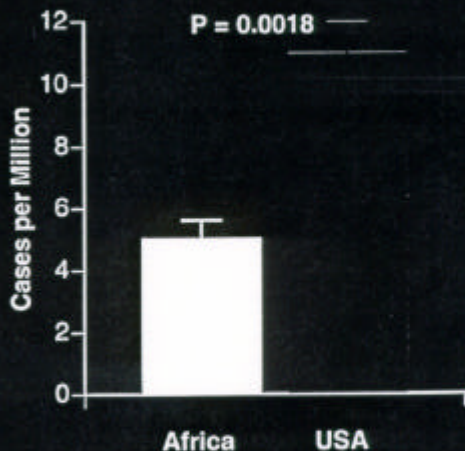
Cancer in Black Children
Africa vs U.S.A.
Those More Common in Africa

Retinoblastoma

P = 0.03

Cases per Million (y-axis: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20)

Africa   USA

Burkitt's Lymphoma

P = 0.21

Cases per Million (y-axis: 0, 5, 10, 15, 20, 25, 30, 35)

Africa   USA

All Soft Tissue Tumors

P , NS

Cases per Million (y-axis: 0, 10, 20, 30, 40, 50, 60)

Africa   USA

Kaposi's Sarcoma

P , NS

Cases per Million (y-axis: 0, 10, 20, 30, 40, 50, 60, 70, 80)
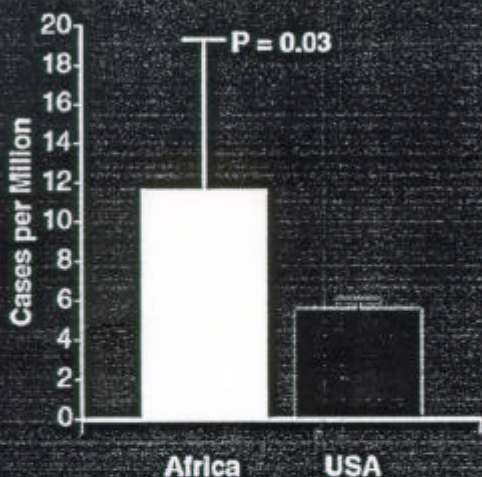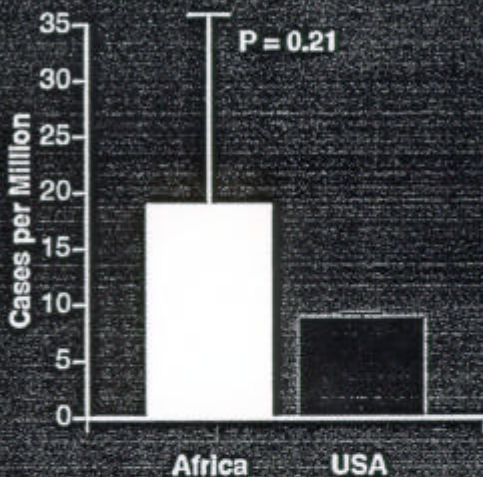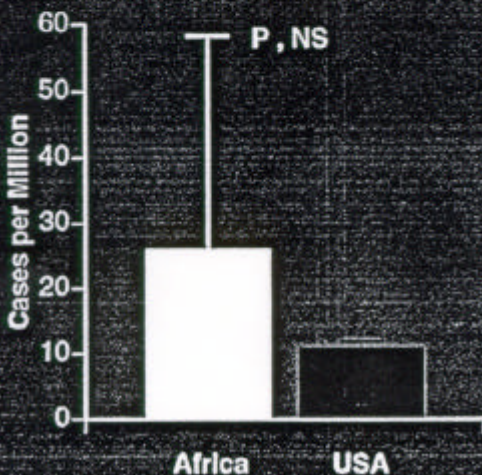
Africa   USA
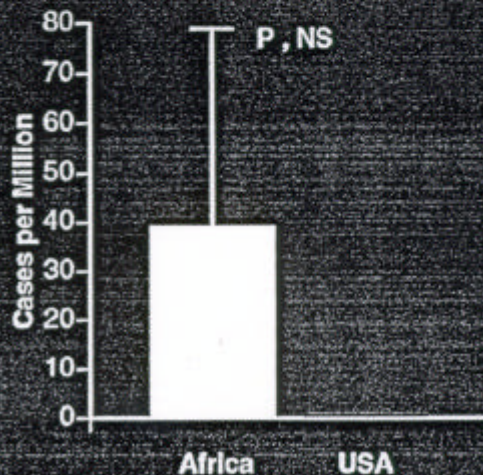
For all cancers, there is really no striking difference between black children in Africa and those in the U.S. That is true also for some individual types of cancer, such as Hodgkin's and non-Hodgkin's lymphoma, kidney tumors and all carcinomas. However, there are some types of cancer that appear to be significantly more frequent in black children in the United States, including brain/neuroblastoma, leukemia, and all soft tissue cancers except for Kaposi's sarcoma. It is interesting that this includes the two tumor types that have been suggested to be increasing in the United States, brain and leukemia. The cancer data are perhaps most uncertain for brain, where you do not have a palpable mass, whereas for leukemia, on the other hand, this difference is supported by data from other countries such as Japan and India. As modernization arrives with all of its pluses and minuses including hygiene and reduction in infant mortality, the risk of leukemia increases.

Infection-related cancers, not surprisingly, are higher in African black children. Burkitt's lymphoma and Kaposi's sarcoma, and interestingly, and I think unexpectedly, retinoblastoma appear to be more frequent in African black children.

Possibly causation of childhood cancers, especially leukemia, nervous system, and soft tissues

What?

1. Diet
   - Nutrients
   - Contaminants

2. Pharmaceuticals

3. Occupational exposures

4. Environmental exposures

5. Lifestyle factors/exposures

6. Infectious agents

To me, this indicates that there is at least possibly a causation or potentiation of childhood cancers related to the environment, at least for those of certain types. If this is so, what might be the causes? We have a long list of candidates. These include the diet (including both nutrients and contaminants), pharmaceuticals, occupational exposure, environmental exposure, and all the lifestyle factors, such as alcohol, tobacco, infectious agents, etc. How are we going to sort these out?

Traditional Epidemiology

Cancer as the Endpoint

Molecular Epidemiology $\Longleftarrow$ **Experiments in Animals**

- Biomarkers
- Genetic changes in tumors
- Susceptibility genes

- Identify risks
- Confirm biological plausibility
- Study the mechanisms
- Generate hypotheses
- Test preventive strategies

There are three investigative arms. We have traditional epidemiology with cancer as the endpoint, and more recently molecular epidemiology with biomarkers, genetic changes in the tumors and susceptibility genes; these are the best approaches of course because they are looking directly at the human, but there are serious limitations with these. Also, experiments in animals have their place for identification of risks to confirm biological plausibility, study carcinogenic mechanism, generate and test hypotheses, and to test preventive strategies.

Which time periods in development have greatest susceptibility to tumor initiation?

Exposure periods

- Preconceptional

- Embryo/fetus (transplacental)

- Neonate/infant (transmammary or direct)

I am going to be proceeding in this context to try to look at the specific questions that were posed for this symposium by the organizers. The first one being which time periods in development have the greatest susceptibility to tumor initiation, in other words, the issue of whether there are windows of susceptibility. We have recently participated in a review on this subject, which is coming out in Environmental Health Perspectives (EHP) next month. There are three broad exposure periods, the preconceptional, the embryo and the fetus by the placenta, and the neonate and the infant either transmammary or direct.

Stages of Susceptibility for Preconceptional Carginogenesis

* Positive results for all stages from

  - fetal gonocytes to
  - mature sperm/eggs

* Most sensitive: postmeiotic sperm, where DNA repair capacity is lacking

* Agents: urethane, diethylnitrosamine, chromium (III), benzpyrene, dimethylbenzanthracene, DES, X-rays, Y rays, and neutron/y rays

* Outcome: tumors characteristic of the species

  - lung, liver, lymphoid in mice
  - upper respiratory tract in hamsters

FATHERS SHOULD NOT BE IGNORED AS POSSIBLE TARGETS FOR RISK FACTORS IN PERINATAL CARINOGENESIS.

With regard to preconceptional carcinogenesis, in animal models there have been positive results for all stages, from the fetal gonocytes of the individual that will eventually become the parent to the mature sperm and the eggs.

For the sperm, the most sensitive stage appears to be the postmeiotic where DNA repair capacity is lacking. That is all that we know about the mechanisms of preconceptional carcinogenesis. The mechanism is probably novel; it may be epigenetic in nature; this is something that we're actively studying. A wide variety of agents have been shown to be effective in animal models, different types of carcinogens, organic and inorganic, DES and several types of radiation.

It is important to note that the type of outcome in terms of the tumors usually is characteristic of the species and strain. For example, tumors of the lung, liver and lymphoid tissues predominate in mice, but tumors of the upper respiratory tract are most common in hamsters. Therefore, we cannot necessarily predict from the type of tumor in an animal model what could be happening in the human. I think a very important implication from this is that fathers should not be ignored as possible targets for risk factors in perinatal carcinogenesis, and they've been barely mentioned so far in this meeting.

Which time periods in embryonic/fetal/infant development have greatest susceptibility?

- Humans: Not known, nor likely to be known, for most exposures.

- Animal models: Basic factors influencing susceptibility during development

* Numbers of cells at risk

* Rate of cell division
  - Sensitivity to killing
  - Sensitivity to mutation

* DNA repair capacity

*Clonal expansion of initiated cells

* Degree of differentiation
  - Enzymes for activating/detoxifying carcinogens
  - Other unknown factors

* Maternal/placental metabolism

* Presence of stem cells

To underscore this point, parenthetically, I want to show this slide

Hot Dog Consumption and Childhood Leukemia
L = Low   M = Medium   H = High

Odds Ratio (Adjusted)

p=0.01

p=0.01

L M H
Fathers

L M H
Children

L M H
Mothers

Fathers and
Children
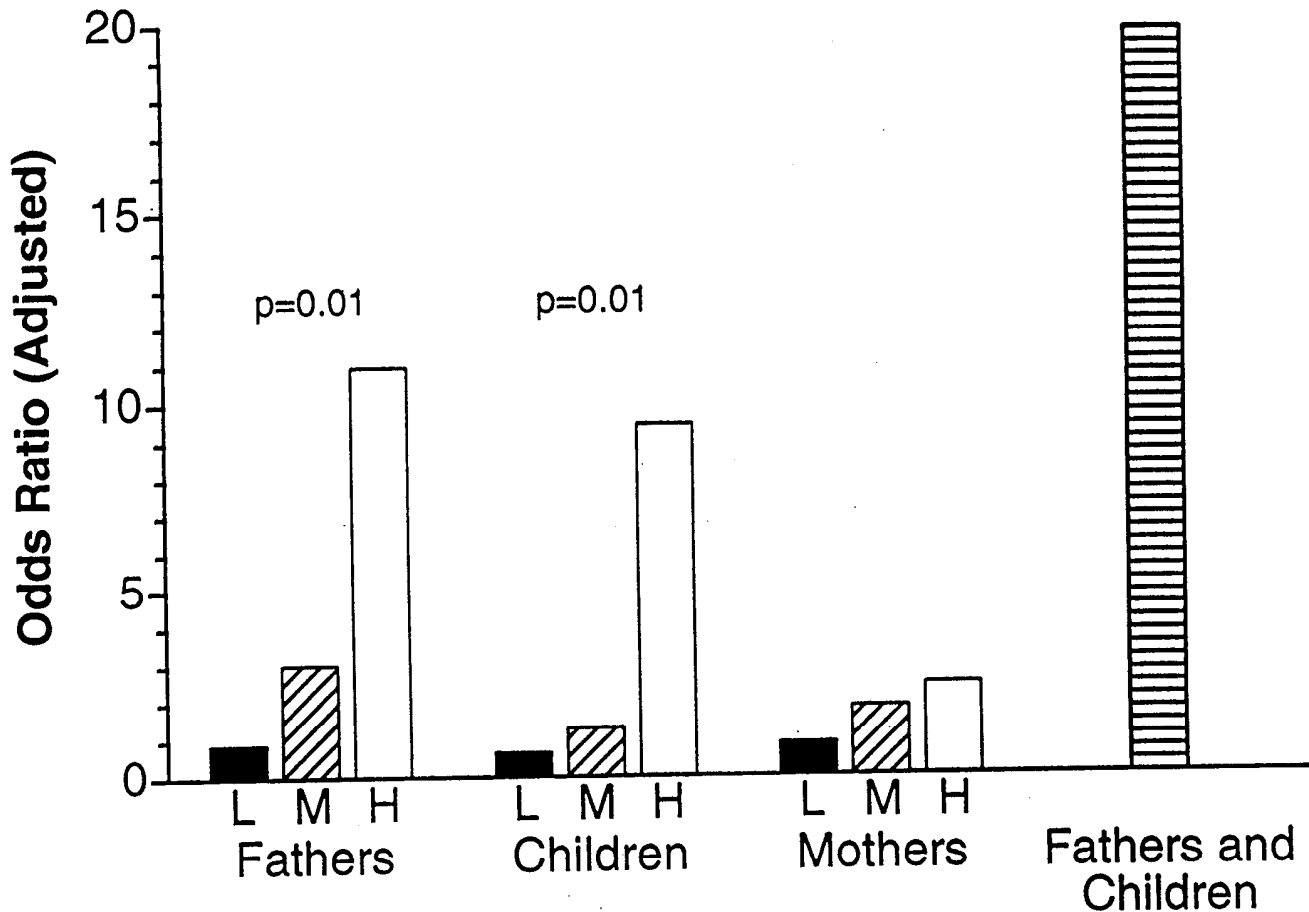
from a very reputable epidemiology group here in California who are looking at hot dog consumption and childhood leukemia. Hot dogs are of interest because they contain nitrates, precursors of carcinogenic nitroso compounds, and cured meats have been implicated before in perinatal carcinogenesis. As shown in Slide 10, there was a highly significant dose-dependent effect with fathers' consumption of hot dogs, and with children's consumption of hot dogs. Whereas, the mother's consumption has very little effect. If both the fathers and the children were high consumers, there was an additive and quite a high risk. I am not trying to implicate hot dogs, or indict hot dogs *per se*, since this is just one study. It is being taken seriously. I know somebody that has a National Institutes of Health (NIH) grant to characterize the nitrosamines present in hot dogs.

What I am trying to emphasize is that the fathers and the children may well be particularly sensitive targets, mothers are tough and should not be focused on to the exclusion of the fathers who are a lot more fragile. (*Laughter*)

Factors in developmental sensitivity to tumor initiation: Correlation Studies

FACTOR	MOST SENSITIVE TIME	ANIMAL MODEL RESULTS		
		#SPECIES	# TISSUES	# AGENTS
Cells at risk	Proportional to size: late fetus, infant	1 (mouse)	2 (skin, lung)	3 (DBMA, radiation, ENU)
Cell division/ killing	Early embryo Late fetus	1 (mouse 1 (rat)	- 1 (brain)	1 (urethane) 1 (ENU)
Cell division/ mutation	Early embryo Early organogenesis	3 (mouse, rat, hamster)	2 (lung, nervous system)	1 (ENU
DNA repair	Neonate	1 (mouse)	2 (lung, liver)	1 (NDMA)
Clonal Expansion	Early organogenesis	1 (mouse)	2 (lung liver)	1 (ENU)

For humans the exposure is pretty much continuous for all the stages. But from animal models there is a pretty good indication that basic factors influencing susceptibility can show variability during development. These include the numbers of cells at risk, rate of cell division which affects sensitivity to cell killing, sensitivity to mutation, DNA repair capacity, clonal expansion of initiated cells, degree of differentiation, which includes enzymes that activate or detoxify carcinogens as well as other unknown factors, maternal placental metabolism, and the presence of stem cells.

Factors in developmental sensitivity to tumor initiation: Correlation Studies

FACTOR	MOST SENSITIVE TIME	ANIMAL MODEL RESULTS		
		#SPECIES	# TISSUES	# AGENTS
Carcinogen* Activation	Late fetus	3 (mouse, rat hamster)	4 (lung, liver, nervous system, trachea)	4 (3-MC, DMBA, NDMA, NDEA)
Carcinogen detoxification	Neonate	1 (mouse)	1 (lung)	1 (urethane)
Placental metabolism	Early gestation	1 (mouse)	1 (lung)	1 (methylnitrosour ethane)
Stem cells	Perinatal	4 (moue, rat, rabbit, oppossum)	1 (kidney)	2 (ENU, MNU)

There are three main points I want to make on the next couple of slides (Slides 12-13). One is that all these factors are rooted in basic biology, and there is no reason to think that they should not also pertain to the human. However, all of the studies with the exception of one that I will come to in a minute are correlation studies and, so, they do not constitute proof. Furthermore, most of these factors have been studied only in one species or in one tissue or with one agent, which provides flimsy groundwork for extrapolating to the human.

The number of cells at risk of course are going to be proportional to size, which will be higher in the late fetus and the infants than in early developmental periods. Cell division and sensitivity to killing has been shown for the early embryo and the late fetus in different situations. There is evidence for sensitivity to mutation for both the early embryo and early organogenesis. With

respect to DNA repair in the neonate, I will show you an example of this in a minute. For clonal

expansion, the most sensitive time is during early organogenesis.

**CO-EXISTING FACTORS FOR SUSCEPTIBILITY TO
PERINATAL TUMOR INITIATION**

Early Embryo/early organogenesis

- Rapid rate of cell division, sensitivity to killing and to
mutagenesis
- High proportion of stem cells
- Opportunity for clonal expansion of mutated cells
- Limited placental development of detoxification protection

Late fetus/neonate

- More target cells
- More differentiated, including carcinogen-activating enzymes

Neonate/infant

- More target cells
- More differentiated, including carcinogen-activating enzymes
- Lack of maternal and placental detoxification protection

The one factor for which the evidence is perhaps strongest involves the ontogeny of the

cytochrome P450 enzymes that activate carcinogens. This has been shown by correlation with

the most sensitive time in the late fetus for three species, four tissues and four agents. We have,

furthermore, confirmed this by genetic manipulation studies.

Other factors include carcinogen detoxification studied in the neonate, placental metabolism in

detoxification in early gestation, and stem cells with regard to kidney tumorigenesis that are

important around the time of birth.

SUSCEPTIBILITY AT SPECIFIC DEVELOPMENTAL STAGES IN ANIMAL MODELS

- Integrated result of the interplay of probably at least nine different mechanistic factors.

- Some of these can be influenced by genetics, other exposures, etc.

- Specifically determined by species/strain, target tissue, and exposure agent.

CONCLUSION FOR THE HUMAN

- There are probably stage-specific differences.

- Detailed empirical information will be required to identify these; this will be difficult if not impossible to get.

Obviously these factors can coexist so it is difficult to tease out just which one is the important one in any particular situation. Thus, in the early embryo, and to an extent in early organogenesis, you have both the high rate of proliferation and the high proportion of stem cells, as well as the opportunity for clonal expansion and limited placental development for detoxification for the early embryo, whereas in the late fetus and the neonates you have more target cells, and a more differentiated state. Also, in the infant and the neonate you have these two, plus you have lost the maternal and placental detoxification protection that was afforded to the fetus.

CAN DEVELOPMENTAL EXPOSURES INFLUENCE VULNERABILITY TO TUMORIGENESIS LATER IN LIFE?

Yes, though little studied; both positive and negative effects.

Susceptibility at specific stages is going to be the integrated result of interplay of probably at least nine different mechanistic factors. Some of these can be further influenced by genetics and other exposures and so forth, and can be specifically determined by the species and the strain, the target tissue and the exposure agent.

For the human, I think there are probably stage-specific differences but it is going to require detailed empirical information to identify these. I think this is going to be pretty difficult information to obtain.

**Developmental Exposures Influencing Later Vulnerability
Mammary Tumors**

Perinatal Exposure		Postnatal Exposure	Outcome
Stage/species	Agent		
Gestation day 14, hamsters	DES	DMBA	Increased incidence of mammary, ovarian, uterine, and melanoma tumors
Neonates, rats	Testosterone Estradiol Prolactin Genistein	DMBA	Decreased Mammary tumors
Gestation, rats	Diet high in polyunsatruated fats	DMBA	Increased Mammary tumors
Transplacental/ translactational, rats	Heterocyclic amine food mutagen PhIP	PhIP	Synergistic increase in mammary tumors
Paternal preconception, rats	X-ray	Urethane	Decreased mammary tumor latency

Rustica and Shubik, 1979
Mori, et al., 1980
Lamartiniere, et al., 1998
Hilakivi-Clarke, et al., 1997
Hasegawa, et al., 1995
Vorobtsova and Kitaev, 1988

Another important question is: Can developmental exposures influence vulnerability to tumorigenesis later in life? The answer to this comes from animal models and is yes, although it has not been well studied; both positive and negative effects have been reported.

Developmental Exposures Influencing Later Vulnerability
Lung and Liver tumors

Perinatal Exposure		Postnatal Exposure	Outcome
Stage/species	Agent		
Gestation day 0-8, mice	X-ray	Urethane	Increased lung tumors
Preconception as gestation day 15 female or day 9 male mice	X-ray	Urethane	Increased lung tumors
Preconception, male mice	X-ray	Urethane	Increase lung tumors
Preconception, male and female rats	Y-ray	NDEA	Increase liver foci
Transplacental	3-MC	NDEA	Reduced liver tumors

There have been a number of studies with the mammary gland where perinatal exposures have included preconceptional, gestational, and neonatal to hormones or hormone-like agents, diets, carcinogens, and x-rays. In adult life exposure to carcinogens like DMBA, or PhIP or urethane, in most cases, has resulted in an increase in the incidence of the mammary tumors or a decreased latency. But in a study with hormones or hormone-like agents there was a decrease in the mammary tumors.

**DEVELOPMENTAL EXPOSURES AND LATER
VULNERABILITY**

Mechanisms: Unknown

Possible mechanisms:

- Permanent alterations in hormones

- Alterations in carcinogen-metabolizing enzymes

- Alterations in immune response/inflammatory
    processes

- Genetic damage contributing part of that needed for
    tumorigenesis

Possible influence on human risk: Yes

Worth further study: Yes

This topic has also been studied with the liver and the lung as an endpoint. Again animals were

subjected to exposures preconceptionally or during gestation to radiation or a carcinogen, and

then postnatally to a variety of carcinogens (such as urethane, NDEA), which increased tumors

in most cases but in one case reduced the number of tumors.

**WHAT IS KNOWN ABOUT INTERACTIONS OF TOXICANTS,
WITH REGARD TO PERINATAL TUMOR INITIATION?**

* Induction of cytochrome P450 1A1 potentiated tumor initiation by methylated
polycyclic aromated hydrocarbons 3-MC and DMBA (but not BP) in mouse fetuses.

This same induction in their mothers protected against tumor initiation in the fetuses.

<div align="right">Anderson, et al., 1985, 1989, 1995</div>

*BHA protected against transplacental carinogensis by DMBA in mice.

<div align="right">Rao, 1992</div>

*Transmammary polychlorinated biphenyls reduced liver tumors initiated by N-
nitrosodimethylamine. However, the bioretained PCBs promoted tumors which were
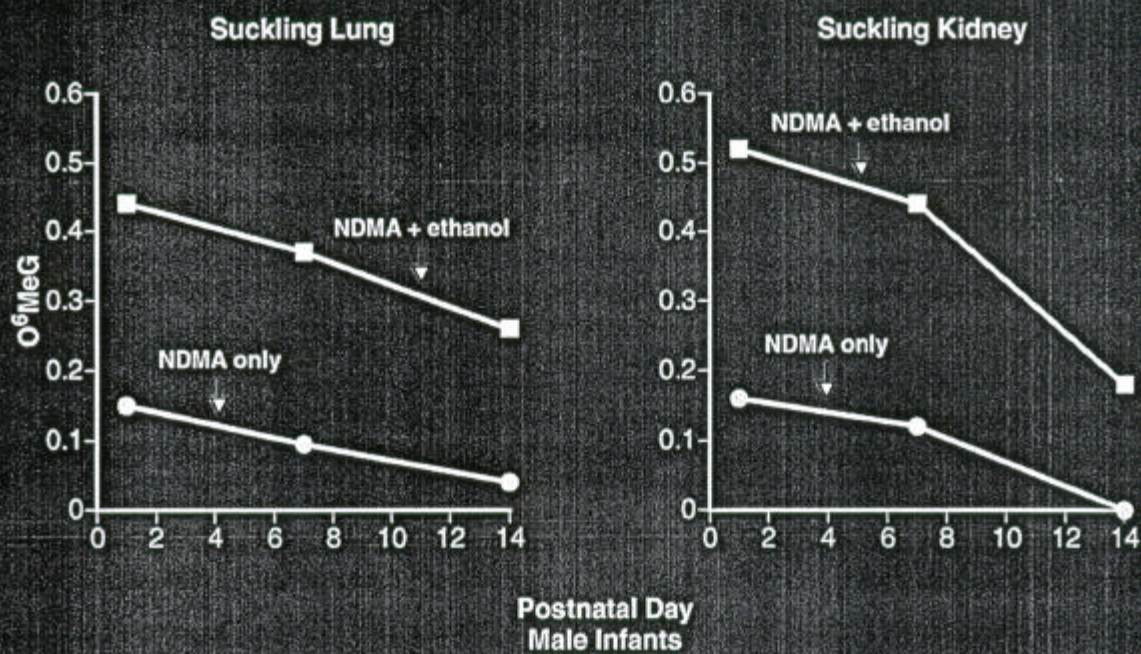initiated.

<div align="right">Anderson, et al., 1983</div>

*Ethanol given to pregnant hamsters with the tobacco carcinogen NNK increased
tumors of pancreas and upper respiratory tract in offspring.

<div align="right">Schuller, et al., 1993</div>

Mechanisms were not studied with any of these models so we do not really know what they are.
Possible mechanisms include alterations in hormones, which is very likely for those situations
where there was hormone exposure perinatally, and possibly alterations in metabolizing
enzymes, in immune response, or in inflammatory processes. Also, it could be that genetic
damage has occurred, which contributes part of what is needed for the full tumorigenic process
that is added onto later. Therefore, there is very possibly an influence on human risk, and I think
this is worth further study because obviously it has implications for exposure in adulthood
following upon perinatal exposures.

Transmammary NDMA and Ethanol in Rats
O^6-methylguanine in DNA

Suckling Lung · Suckling Kidney

O^{6}MeG vs Postnatal Day, Male Infants. NDMA + ethanol; NDMA only.

IDENTIFICATION OF THE MOST SUSCEPTIBLE
SUB-GROUPS OF CHILDREN

1. Animal models suggest that the newborn is the most
susceptible of all immature states.

2. Animal models predict that polymorphisms in
cytochromes P450 and other carinogen-metabolizing enzymes
may influence risk.

> * High expression in the fetus may increase risk.
> * High expression in the mother may decrease risk.

What about the interactions of toxicants? Again, there is some information indicating some

complex effects. We found that induction in the cytochrome P450 isozyme that activates

polycyclic aromatic hydrocarbons (PAHs) potentiated tumor initiation in mouse fetuses by

methylated PAHs, like 3-methylcholanthrene (3-MC) and DMBA, but interestingly not by

benzo[a]pyrene, so to lump compounds together here would be a problem. Furthermore, the

same induction in their mothers, along with phase-two induction, protects against tumor

initiation in the fetuses. Thus, we found that the highest risk was of inducible fetuses being

carried by a non-inducible mother, whereas, the lowest risk was for non-inducible fetuses being

carried in an inducible mother.

Other interactions include the following. BHA protected against transplacental carcinogenesis

by DMBA. We found that transmammary PCBs reduced the liver tumors that were initiated in

infant mice by dimethylnitrosamine, but those that were initiated were promoted by the

bioretained PCBs in the offspring, so they grew bigger and were more malignant. Ethanol given

to pregnant hamsters with a tobacco nitrosamine (NNK) increased the tumors of the pancreas and

upper respiratory tract in the offspring.

**DO ANIMAL MODELS ADEQUATELY PREDICT
HUMAN RESPONSE?**

X-Radiation, transplacental

- leukemia in children

- lymphoma/leukemia in mice, but only after postnatal
treatment

> * and lung and ovarian tumors after
> transplacental treatment

DES, transplacental

- adenocarcinoma of vagina in humans, selectively

- tumors of lower reproductive tract in mice, selectively

Another example of ethanol-carcinogen interactions comes from new data from my lab. Rats

were given dimethylnitrosamine during lactation on either the first, the seventh or the 14th day

after parturition. Some of them were given ethanol at the same time at a high-human-

intoxicating dose. The infants were allowed to suckle for four hours and then the tissues were

taken. Dr. Souliotis in Athens measured the O^6-methylguanine in the DNA, which is a

promutagenic adduct that has been definitely linked to tumor initiation. There are a couple of

interesting points here. One is that the highest level of adducts in these tissues (lung and kidney,

and also the liver), was in the neonates on day one after birth, and decreased thereafter,

dramatically so in the kidney. Other studies have shown that this correlates perfectly with the

ontogenic appearance of the enzyme that repairs this lesion. This is an example of neonatal

sensitivity due to a lack of the DNA repair enzyme.

The second interesting finding is that the amount of adducts are increased four or five times

when the NDMA was given together with the ethanol. This is almost certainly due to the fact

that the ethanol inhibits the hepatic first-pass clearance of NDMA in both the maternal liver and

the infant livers, giving a large interactive effect here.

QUANTITATIVE COMPARISON OF DES EFFECTS

Humans

Median total dose: 10 g, ~ 150 mg/kg
Minimal effective total dose: 10 mg/kg
Minimal effective daily dose: 0.023 mg/kg

Estimated risk: 0.1% (to age 34)

Mice

Total dose: 1mg/kg
Minimal effective total dose: 0.25 mg/kg
Minimal effective daily dose: 0.0025 mg/kg

Risk: all reproductive tract tumors: 10-20% (most in old mice)
        Vaginal adenocarinoma: 0.7 - 3%

Mice more sensitive by as much as: 150/1 x 20/0.1 = 30,000
        (All tumors, total dose)

Or as little as: 23/2.5 x 3/0.1 = 280
        (Vaginal adenocarcinomas minimal effective daily dose)

What about the most susceptible subgroups of children from the animal models? A couple of things I think are fairly strongly suggested. The first is the newborn is the most susceptible of all the immature stages. The second is that the animal models predict that polymorphisms in the cytochromes P450 and related enzymes can influence risk, with high expression in the fetus perhaps increasing risk, but high expression in the mother perhaps decreasing it.

DATA GAPS WITH REGARD TO PERINATAL CARCINOGENESIS IN ANIMAL MODELS

1. Confirmation of important susceptibility factors
   * Correlations with several species/chemicals/target tissues

2. Use of special genetic models to address questions/hypotheses from molecular epidemiology

3. Comparison of molecular changes in human childhood cancers and in perinatally-caused animal tumors

4. Stage-specific assays for preconceptional, transplacental, neonatal effects common human exposure agents
   * Pesticides
   * Drugs of abuse
   * Tobacco carinogens
   * Common water and air contaminants
   * Etc.

5. Exploration of multi-exposure effects
   * Simultaneous
   * Sequential

6. Studies of chemoprevention/intervention

Do animal models adequately predict human response? We could look at this for the two examples of human transplacental carcinogenesis, x-radiation in humans leading to leukemia in children, and diethylstilbestrol (DES) in humans leading to adenocarcinoma of the vagina. Exposure of mice to x-rays does give lymphoma and leukemia but only after postnatal treatment. Some effects are seen with infant mice but these effects are more pronounced as the mice get older. Transplacental exposure of mice gives lung and ovarian tumors instead. Therefore, while the infant mouse might be somewhat similar to the human fetus, I think the predictive value here is rather problematic.

For DES, however, there is good qualitative predictivity, where you get adenocarcinoma of the vagina selectively so far in humans, whereas, in mice you get tumors of the lower reproductive tract selectively.

**IMPROVEMENT IN CANCER RISK ASSESSMENT
USING CURRENT INFORMATION**

1. Global formulae will not work. Every specific risk situation
    will need detailed evaluation.

2. Animal model results could be used to greater effect.

3. Fathers should receive more attention.

You can also make a quantitative comparison of the DES effects. For humans total doses and

minimal effective total and daily doses have been estimated and a risk to age 34 of about 0.1%.

You can see that the mice got a much lower dose, and their risk was considerably higher,

depending on whether you look at all tumors or the vaginal tumors. These are mostly in the old

mice, and we still don't know what's going to happen in the old humans. Depending on how you

calculate it, the mice so far appear to be between about 300 to 30,000 times more sensitive,

quantitatively, than the human.

There are data gaps with regard to perinatal carcinogenesis in animal models. There are

experiments that could be done in animal models that might help risk assessment. As I

mentioned, these important susceptibility factors varying during development could be

confirmed by more studies with several species, chemicals and target tissues. We now have the

capacity to make special genetic models, for example transgenics and knockouts, to address

questions and hypotheses that come from human molecular epidemiology.

It could be useful to compare the molecular changes in human childhood cancers, which are increasingly understood, with those in perinatally-caused animal tumors, because if they are similar it will greatly increase our assurance that these are predictive of human risk.

My personal opinion is that we need more stage-specific assays for a variety of human exposure agents, for preconceptional, transplacental and neonatal effects. This has been mentioned a couple of times already at this meeting. The tendency has been to simply tack perinatal exposure onto a two-year study. In a lot of cases I do not think this is adequate because there can be qualitative differences and differences in dose response. Postnatal exposures, particularly to high doses, can suppress the effects of perinatal exposures or mask them out.

I think animal models could be used more for exploration of multi-exposure effects, both simultaneous and sequential.

Finally, chemoprevention and intervention investigations are well underway with adult animal models -- this has been barely touched on in the perinatal context.

Finally: What can be suggested for improvements in cancer of risk assessment from the current information? I could not really think of very much that would be of great specific use. I think it is evident from the complexities involved that global formulae are not going to work. Every specific risk situation is going to require detailed attention. Maybe the new technologies in genetics and so forth are going to do this; I do not know that I'm quite as sanguine about this as Chris was this morning.

I think animal model results could be used to greater effect. My observation is that many epidemiologists and many risk assessors do not have a working knowledge of this animal literature and may not even be aware of it. Finally, let me emphasize again that I think that the fathers, the delicate men, should receive more attention.
Thank you.